7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com;
Email: info@abpbio.com

MycoCheck™ Mycoplasma Stain Kit

Catalog Number: A059-1, A059-2

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
MycoCheck™ Mycoplasma Stain Kit (Cat. No. A059-1)			The product is stable for at least 6 months when stored as directed.
MycoFluor Reagent (Component A)	2 mL	2-8 °C Protect from light	
Antifade Mounting Medium (Component B)	2 mL		
MycoCheck™ Mycoplasma Stain Kit (Cat. No. A059-2)			
MycoFluor Reagent (Component A)	10 mL	2-8 °C Protect from light	
Antifade Mounting Medium (Component B)	10 mL		

Product Description

Mycoplasma infections are relatively common in laboratory cell cultures; it has been estimated that between 5% and 35% of all cell cultures are infected. Mycoplasmas have been shown to alter the growth rate of cells in culture, induce chromosomal aberrations, influence amino acid and nucleic acid metabolism and cause membrane aberrations. Several methods have been developed to detect mycoplasma including direct culture in special growth media, enzyme-linked immunoassay, immunofluorescence staining, PCR, biochemical detection and fluorescent nucleic acid stains.

The MycoCheck™ Mycoplasma Stain Kit provides an ultrasensitive, rapid and simple fluorescence microscopic assay for the visual identification of mycoplasma infection in laboratory cell cultures. In order to detect mycoplasma, the fluorescent MycoFluor reagent is added directly to the culture medium, with or without cells, and the stained sample is then examined under a fluorescence microscope. The excitation and emission spectra of MycoFluor reagent bound to dsDNA are similar to dsDNA bound to DAPI, with excitation maxima around 350-360nm and emission maxima around 450-460nm. MycoFluor reagent can be excited either with a xenon mercury arc lamp or a UV laser and is detected through a blue filter.

Mycoplasma staining with MycoFluor reagent appears as a fine particulate or filamentous staining over the cytoplasm at 100X magnification. Nuclei of the cells are also brightly stained by this method and thereby act as endogenous positive control for the staining procedure.

Materials required but not provided in the kit:

- Sterile tissue culture grade water
- Sterile PBS buffer
- Carnoy's fixative (3:1 Methanol: Glacial acetic acid)
- Centrifuge tubes
- Glass slides
- Microscope coverslips

General Protocol

The kit has been designed to detect Mycoplasma in both suspension (non-adherent) and adherent cell cultures.

1. Staining of suspension cells

1.1 Aseptically aspirate the culture medium containing suspension cells from the culture vessel and transfer it to a sterile centrifuge tube.

1.2 Centrifuge the tube at 1000rpm for 10 minutes at room temperature.

1.3 Discard the supernatant and resuspend the pellet in 500µl of medium.

1.4 Add 1ml of freshly prepared Carnoy's fixative and mix well.

1.5 Centrifuge at 1000rpm for 10 minutes at room temperature.

1.6 Discard the supernatant and resuspend the pellet in 500µl of sterile PBS buffer.

1.7 Apply one drop of MycoFluor reagent (Component A) and mix well.

1.8 Allow it to stand for 15-20 minutes at room temperature, in dark.

1.9 Add one drop of the suspension on clean, greasefree slide and make a thin smear. Allow it to air dry.

1.10 Apply one drop of anti-fade mounting medium (Component B) on the smear and put a coverslip on it.

1.11 Observe the slide under fluorescence microscope.

2. Staining of adherent cells

Cells should be grown at 50-80% confluent before use. Cells could be grown on slides or chamber slides or on coverslips in Petri dish or 6-well tissue culture plates.

2.1. Aspirate the medium from culture vessel

2.2. Add sufficient volume of freshly prepared Carnoy's fixative to cover the monolayer completely.

2.3. Allow it to stand for 10 minutes at room temperature.

2.4. Remove the fixative.

2.5. Add 1ml of sterile PBS buffer, then apply one drop of MycoFluor reagent (Component A) and mix well.

2.6. Allow it to stand for 15-20 minutes at room temperature in dark.

2.7. Remove the left over stain solution and allow it to dry.

2.8. Mount the slide/coverslip as follows:

a. Slide - Apply one drop of anti-fade mounting medium (Component B) on the upper cell sheet surface of the slide and cover with a coverslip.

b. Coverslip - Apply one drop of anti-fade mounting medium (Component B) to a glass slide. Put the coverslip on the mounting medium with cell side down.

2.9. Observe the slide under fluorescence microscope.

Interpretation of the results:

1. Points to be considered before interpretation of the results:

a. The slides should be observed under a fluorescence microscope using objectives of magnification 40X or 100X. They can also be observed using a 100X oil immersion objectives.

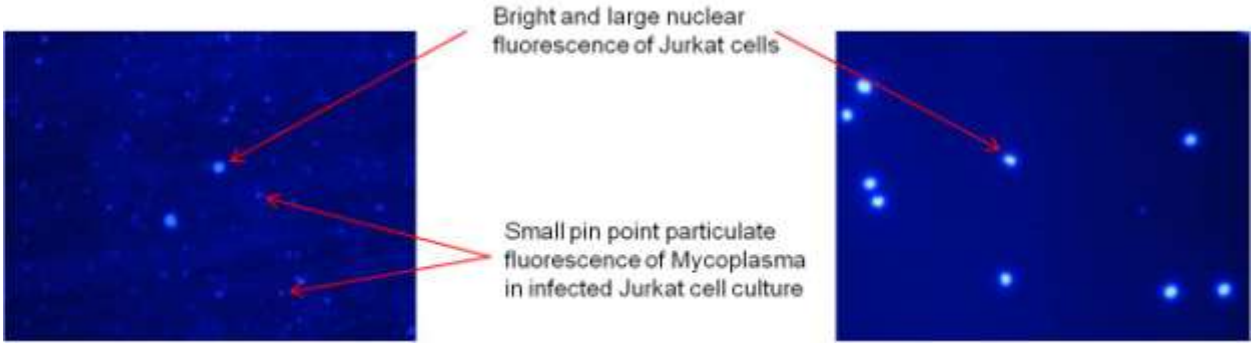
b. To observe the specimens stained with MycoFluor reagent, excitation and emission filters of wavelengths 340-380nm and 490-510nm, respectively, must be used.

2. Interpretation:

• If the culture is negative for Mycoplasma then it will show only nuclear fluorescence. Occasionally micronuclei or nuclear fragments from dead and disrupted cells will appear as spherical bodies. Their large size and brighter fluorescence will distinguish them from Mycoplasma.

7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com;
Email: info@abpbio.com

• If the culture is positive for Mycoplasma then along with nuclear fluorescence it will also show extra-nuclear fluorescence. Mycoplasma can be identified by small pin point dots of fluorescence, either aggregated in clusters or scattered uniformly over the cytoplasm and sometimes in the intercellular spaces.



3. General guidelines for interpretation:

a. Bacteria, yeast and other prokaryotes show typical size, morphology and growth characteristics (i.e. chains, budding, mycelia, etc.).

b. Complete scanning of the specimen slide or test slide is necessary before interpreting the results because all the cells may not be infected with *Mycoplasma*. Incomplete scanning may result in false negative results.

c. If there is any doubt regarding the interpretation of the fluorescence, test should be repeated after generating a further subculture of the test cells in absence of antibiotics.

d. Further confirmation of the *Mycoplasma* infection can be done using other assays such as PCR, ELISA or direct growth on agar or in broth.